Immunoglobulins

Uptodate: Immunology

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University of London Audio-Visual Centre, 1974.
Made for British Postgraduate Medical Federation.

Produced by Peter Bowen and David Sharp.

Black-and-white
Duration: 00:42:41:11

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<Opening titles>

<Dr Turner to camera>

The immunoglobulins are a family of related proteins essentially involved in the immune response of all higher vertebrates. Like many plasma proteins, they were first described as a result of observations upon their function, which is, of course, the possession of antibody activity. It was Emil von Behring, shown in this first picture, […]

<Turner refers, in turn, to portrait photographs and then a diagram displayed on stand. Narrates over these using indicator stick, interspersed with talk to camera>

[…] who described in 1890 the production of antitoxins in the serum of guinea pigs immunised with the exotoxin of the diphtheria bacillus. But detailed chemical characterisation only became possible when Tiselius, shown in this picture, described the technique of electrophoresis for separation of serum proteins, and with

Kabat in 1939 successfully demonstrated that the antibody activity of serum resided in the so-called gamma globulin region of the serum.

<Turner to camera>

After the Second World War, ultracentrifugation and immunoelectrophoretic techniques were used to show that the gamma globulin fraction was indeed even more heterogeneous, and by 1959 there was sufficient evidence for Heremans to suggest that a family of proteins existed and to which the term immunoglobulins could be applied.

<Turner narrates over slide showing list of immunoglobulin types>

The next table shows that we recognise, today, five proteins as belonging to the immunoglobulin system. These are called immunoglobulin G, A, M, D and E. And for shorthand use, we call them IgG, IgA, IgM, IgD and IgE. Previously, the IgG fraction has been commonly referred to as the 7S gamma globulin fraction, and the IgM as the 19S gamma globulin fraction.

<Turner narrates over slide showing physicochemical characteristics of immunoglobulins>

The next table shows some of the physicochemical characteristics of these proteins and we could see straight away that there's considerable heterogeneity. For example, the molecular weights of the proteins varies from 160 000 for IgG and IgA up to 900 000 for IgM, with the IgA of secretions having a molecular weight of about 385 000, and IgD and IgE both having molecular weights slightly under 200 000. All the proteins are glycoproteins, that is, they contain carbohydrate and the amount varies from about 3% for IgG up to 12% for IgD. And finally, this table shows that each of the proteins is associated with a special type of polypeptide chain. And we give this a Greek letter: γ for IgG, α for IgA, μ for IgM, δ for IgD and ε for IgE.

<Turner narrates over slide listing biological characteristics of immunoglobulins>

The next slide shows that we have also a heterogeneity in the biological characteristics of these proteins. First of all, if we look at IgG, we see that this accounts for about 70% of the serum immunoglobulin fraction. Most of the antibodies to viruses and bacteria are present in the IgG fraction, but we think that it is particularly important as an antitoxin because of its ability to pass freely between the intravascular and extravascular spaces of the body. The IgG class is also distinguished by being able to fix complement, bind to macrophages, cross the placenta, but it does not bind to mast cells. The IgA class accounts for about 20% of the serum immunoglobulin fraction and, as you can see in the second line of the table, it is devoid of all the so-called effector functions, from complement fixation through to binding to mast cells.

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The IgA of serum is probably less important than the IgA of secretions. This type of IgA is called secretory IgA and is present in parotid secretions, gastrointestinal secretions, bronchial and nasal washings, colostrum and milk. And we think that this is particularly important for creating an immune barrier at exposed mucous membrane surfaces.

The IgM class accounts for about 7 to 10% of the immunoglobulins, but it is, nevertheless an extremely important immunoglobulin. In association with cell membrane antigens, it is particularly effective at fixing the complement proteins. And when the C3b component of complement has been generated, this has a binding site for macrophages, and so complexes involving IgM antibody and complement can be readily phagocytosed by the macrophages. And indeed, we find that IgM is present in intravascular spaces of the body in high concentration. It is an efficient agglutinating antibody and it has this ability to bind complement, and these factors taken together suggest that it is the most important antibacterial antibody in the bloodstream, and that it is especially important in enhancing phagocytosis of particulate antigens. We

see that the levels of IgM are notably high following continuous and direct exposure to particulate antigens in toxoplasmosis and congenital syphilis and such like.

The IgD class of immunoglobulin is present in much lower concentrations and only a few antibody activities have been described in association with this class. And no effect or function has been described for the IgD class.

Then finally, on this table, we see IgE which occurs at the lowest concentration and we feel that this immunoglobulin class is responsible for much of the so-called reaginic antibody activity of the serum. This is the type of antibody which is involved in immediate hypersensitivity reactions, the type 1 reaction, and is able to bind to either tissue mast cells or to circulating basophils, and then to combine with a specific antigen or allergen and to elicit the symptoms of immediate hypersensitivity.

<Turner to camera and then narrates over graph charting development of immunoglobulins>

So of the effector functions, we see that IgE is particularly able to bind to the homologous mast cells, but it is not complement fixing, it is not macrophage binding and it does not cross the placenta.

Turning now to the development of the immunoglobulins, we see that before birth the IgG class of immunoglobulin is represented by an increasing concentration such that at birth we have as much immunoglobulin G present in the foetus as in the mother. But after birth, the level of the immunoglobulin drops off fairly rapidly and enters what is sometimes referred to as a physiological trough at about 4 months of age. This is due in part to the catabolism of the maternal IgG but also to the increasing plasma volume which dilutes out the IgG. Thereafter the level of the IgG rises slowly to give adult levels by about the age of 15, and we see that there is a slight sex difference with females having higher levels than males.

IgM is detectable before birth but at very low concentrations and this is foetally produced IgM. After birth the level increases rapidly to reach about 75% of the adult

level by 1 month of age. Thereafter the rise is, again, progressive and slowly reaching adult levels by about 5 years of age with, again, females having higher levels than males. This sudden rise in the level of IgM is thought to be a response to the colonisation of the gut and general exposure to the antigens of the environment. And, in fact, it can be delayed by keeping an infant in a germfree isolator and then at the time that the child is brought out of the isolator, this rise in IgM is again seen.

The other immunoglobulin classes, IgA, IgE and IgD, all rise very slowly throughout childhood and reach adult levels in late adolescence.

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<Turner to camera and then over photograph of Professor Rodney Porter>

Our knowledge of immunoglobulin structure is largely the product of the last 15 years of work. And the man who has done most to start this investigation is Professor Rodney Porter, who is now working at Oxford University. And in 1959, he was able to demonstrate that antibody molecules could be split by the enzyme papain to give active fragments which could then be studied for relation of structure to function.

<Turner narrates over diagram outlining Porter's experiment on splitting antibody molecules>

And the experiment which Porter performed is shown on this chart. Here is a rabbit IgG molecule. After exposure to the enzyme papain, three fragments are obtained and two of these appear to be identical: they have similar molecular weights of about 45 000, and the important point is that they retain the ability to bind to antigen. The other piece is distinct, it is slightly larger, molecular weight of about 55 000, and it was not able to bind to antigen, but it did retain these effector functions: the ability to cross membranes – the placenta, that is, in man; to bind to macrophage; to bind to skin; and to bind complement. And because it could be crystallised, it was called the Fc fragment.

<Turner narrates over diagram of four-chain structure of IgG molecule>

Shortly after this, both Porter and Adelman independently showed that immunoglobulin molecules could be cleaved by reducing agents and polypeptide chains separated by chromatography in dissociating media. And on the basis of these experiments, Porter, in 1962, was able to propose this four-chain structure for the IgG molecule and here we see two so-called light polypeptide chains, linked to two heavy polypeptide chains by disulphide bridges, shown here by the s-s bonding: light chain linked to the heavy chain, light-chain to the heavy chain, and also heavy chains linked to each other. In addition, there were noncovalent bonds between these polypeptide chains, such as hydrogen bonds and electrostatic interactions.

The cleavage of the immunoglobulin molecule by papain is shown by this dashed line here. And you can see that this gives a part of the top which consists of about half of the heavy chains – this is the Fc fragment, which has the effector functions listed here. And the parts that are obtained from the bottom of this molecule are identical, each has a light chain and each has a part of a heavy chain, and these are the Fab fragments. And because this retained antigen-binding activity, it was proposed that the site for such activity would be found somewhere within the Fab region of the molecule.

<Turner to camera and then over electron micrograph of IgG molecule>

Now, do the IgG molecules, in fact, have a shape which resembles this rather schematic diagram? And the answer is that, up to a point, they do. And the next chart here shows an electron micrograph of an IgG molecule in which there appears to be three distinct regions. And it is thought that the central package of material here would be an Fc fragment and the two outer parts would be Fab fragments. And again, we see here three parts. Other investigators have obtained less good pictures of the IgG molecule in which there appears to be a Y-shaped structure, where the arm of the Y, the two arms of the Y represent the Fab regions, and the stem of the Y, the Fc region.

<Turner to camera and then over diagram showing structure of IgM molecule>

The four-chain subunit structure for IgG has now been extended to other classes of immunoglobulin and, as this chart shows, IgM is also based upon the same substructure. Here we see light chains and heavy chains, but, in this case, the heavy chains are of the new type. And in the case of IgM, there is a not a single four-chain subunit but, in fact, five polymerised together through disulphide bonds in the Fc region. And this gives a molecule which has potentially ten antigen-combining sites, although whether or not all of these binding sites can be effective at the same time is a matter for some dispute.

<Turner to camera and then over electron micrographs of the IgM molecule >

We also have electron micrographs of the IgM molecule, which again shows very nicely the Fab regions of the molecule as the two arms of the Y, here, and the Fc part is hidden in this central electron dense area here. These are photographs of mouse IgM molecules obtained by Parkhouse and Dourmashkin at the National Institute for Medical Research at Mill Hill.

<Turner to camera>

The other polymeric immunoglobulin which is attracting a great deal of attention at the moment is the IgA of external secretions. As I have already said, this IgA has a molecular weight of about 385 000. And we now know that it consists of two four-chain subunits in which the heavy chains consist of alpha chains, but, in addition, it is complexed with an additional protein component which is called the secretory component and also a polypeptide chain called the J chain, the function of which is still somewhat unclear.

<Turner narrates over photomicrograph of dimeric IgA>

Now this next picture shows a photograph of dimeric IgA, but I must confess that it is not secretory IgA, it is, in fact, a serum IgA. And what you see is a Fab fragment

here, another Fab fragment here, an Fc region here, then exactly the same thing again with an Fc and two Fabs at the bottom. So the two Fc fragments are polymerised together.

Whether or not this same structure is applicable to the secretory IgA is still uncertain, but some investigators have obtained electron micrographs which show structures of similar type to this. But where the secretory component resides is by no means clear. It has been suggested that the secretory component has a protective function for this type of IgA in that it protects it against enzymatic digestion, which is an obviously important feature for an immunoglobulin molecule in the gastrointestinal tract, for instance. If this were the case, one might expect the secretory component to be located near the so-called hinge regions of the molecule which are known to be vulnerable to enzymatic attack, for example, here and here. But we have no data on this at the moment.

<Turner to camera>

Now, much of our understanding of immunoglobulin structure today hinges upon studies which have been made on so-called homogeneous proteins, such as myeloma proteins and Waldenström macroglobulins. It was shown in the early 1960s by Adelman and Galli, and independently by Putnam, that these proteins are pathological counterparts of the normal immunoglobulins and that the Bence Jones protein, which is characteristically found in the urine of many of these patients, is a pathological counterpart of the normally occurring light chains. This made possible the amino acid sequencing which has been going on ever since 1962 because, obviously, Bence Jones proteins can be obtained in large yield from patients with myelomatosis without any inconvenience to the patient. And we now have available the amino acid sequence of a very large number of Bence Jones proteins or light chains.

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<Turner narrates over diagram showing sequence of a Bence Jones protein>

And I show here the sequence of a single Bence Jones protein, which is a peptide chain of about 214 amino acid residues in length. And several features emerge immediately. There are two so-called intrachain disulphide bridges in this structure. These are shown by the bars here and here between cysteine amino acid residues. When several Bence Jones proteins had been sequenced and the amino acid sequences were compared, a very surprising feature emerged. And this was that within a given subclass, the amino acid sequence was constant from protein to protein for about half of the length, for about 110 of the amino acid residues. And these were the residues at the carboxy-terminal half of the peptide. In the other half of the molecule, in contradistinction, we see the so-called amino acid variability. There were different amino acid residues present at these positions shown by the black spots. And, in particular, there was a great deal of variability in the amino acid residues adjacent to this disulphide bridge here, in these regions here and here, and also to a lesser extent out here. But other residues, as you can also see, were found to be variable.

Now, the areas which show the greatest degree of variability have subsequently become known as the hypervariable regions. And the whole of this segment is known as the variable region of the molecule. And since we know that the light chain is in the Fab fragment that was isolated by Porter, it is logical to presume that the hypervariability is in some way involved in the antigen-binding specificity of the molecule.

<Turner narrates over diagram showing variable regions in the heavy chain and light chain sequence of a myeloma protein>

Now, the work on the light chains has been extended to the heavy chains also and we have, again, a region which shows amino acid variability, which is again called the variable region of the heavy chain, and a so-called constant region. But in this case, the constant region is much longer than it is in the light chain. This is a model which was proposed by Adelman in 1969, based on the complete amino acid sequence of a myeloma protein which he published in that year. And if we look

carefully at this, we can see that there are periodically disulphide bridges occurring along the peptide chain in the heavy chain. And also there are two of them in the light chain. And these divide up the peptide chain very neatly into equal segments. In the case of the light chain, we see two segments; there is the variable part and the constant part of equal length. In the heavy chain, we have a variable part which is, again, of about 110 to 115 amino residues in length, similar to that of the light chain. And there are three equally spaced disulphide bridges in the constant part which divide that constant region up into what are called three constant homology regions. Now, they're called constant homology regions because if one takes the heavy chain and splits it between these disulphide bridges and then places the three segments one below another, and compares the amino acid residues which are present in the different regions, there is a very high degree of sequence homology between them.

Now, Adelman has proposed, on the basis of the recurring disulphide bridges, that the peptide material of the immunoglobulin is folded as a series of compact domains, and that each domain is responsible for a specific function of the molecule.

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<Turner to camera and then over an X-ray diffraction plot of Fab fragment>

Confirmation for the domain concept seems to be coming from very recent X-ray diffraction data which have been obtained on the Fab fragments of a myeloma protein which was able to bind phosphorylcholine determinants. This shows an X-ray diffraction plot of such an Fab fragment in which you can see one piece of peptide chain at the top here, which is coming from the light chain which is in the Fab fragment, and another piece, at the bottom here, which is coming from the heavy chain of the molecule. And you can see that the peptide material is clustered into regions which are electron dense. And there are four of them visible here, and between them, there is a relatively diffuse area, or extended area, where there is not very much peptide material. And in this particular photograph, we have the variable regions on the right-hand side here and the constant regions on the left-hand side,

and the area of the molecule which is binding to the phosphoryl choline group is shown by this dashed line up here.

So the conclusion, which has been drawn from such studies is that the binding site of the immunoglobulin molecule is, indeed, in the Fab region and it is in the variable part of the structure and, moreover, it is at the tip of the Fab fragment itself – the part which actually binds to the antigen being a cleft formed by the variable parts of the light and heavy chains together interacting to create a cleft, rather in the way of enzyme substrate binding clefts.

<Turner to camera and then over diagram of immunoglobulin structure showing linked domains>

Now, on the basis of the domain theory, it is possible to construct yet another model for an immunoglobulin structure, in which we see these linked domains, and we can give a name to each of these domains. Here we see the Fab fragments and here is the Fc fragment. And we see that in the Fab region, the amino-terminal domain is composed of the variable region of the heavy chain interacting with the variable region of the light chain. And then we have a domain which consists of the constant part, the first constant part of the heavy chain interacting with the constant part of the light chain.

And then up in the Fc fragment here, we have two dimeric structures composed of the two heavy chains interacting with each other to give two separate domains. Now, we already have good evidence that antigen binding is a property of this amino-terminal domain here; there is fairly good evidence that binding of this C1q component of complement is a property of this domain in the Fc region, and recent work seems to indicate that binding to the monocyte surface is a property of this carboxy-terminal domain in the Fc region in here.

<Turner to camera and then over diagram showing four subclasses of human IgG>

Now, a further complication of the immunoglobulin story is that each of the classes probably consists of a number of subclasses. We know this to be true, for instance, for IgG and IgA. It is probably true also for IgM and IgD. We do not know at the moment if subclasses of IgE exist. And a certain amount of data is now available on both the structure and function of the subclasses of IgG. And this chart here shows the four subclasses of human IgG and the variation which has been described in terms of the disulphide bridge patterns between the light and heavy chains, and between the heavy chains themselves, of these four different subclasses. For example, we have a light chain which is joining to the middle of the heavy chain in the IgG1 subclass, but in all the other three subclasses, the light chain joins, in fact, just between the variable segment of the heavy chain and the first constant segment. And the number of inter-heavy chain disulphide bridges varies from two in IgG1 and IgG4 to as many as five in IgG3.

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<Turner to camera and then over table listing various effector functions of the IgG subclasses>

So there is certainly structural heterogeneity in these subclasses, and the next table shows that there is also functional heterogeneity. We see, again, the four subclasses listed, and various effector functions of the molecule are listed on the left-hand side. First of all, placental transmission: this seems to be a property of all of the four subclasses, although some workers have doubted the efficiency of IgG2 transport, but careful studies have shown that all four subclasses can cross the placenta. Secondly, all four subclasses appear to be able to react with appropriate rheumatoid factors. In this instance, we're talking about the immunoglobulins acting as antigens; they are the antigens with which the macromolecular rheumatoid factors are reacting.

Heterogeneity first shows itself when we look at complement fixation because we can see here that IgG3 is by far the best of the four subclasses in binding C1q, followed by IgG1 and then IgG2. IgG4 probably does not fix complement at all. Then we have heterologous skin fixation, which refers to the ability of these subclasses to bind to

the skin of guinea pigs and elicit passive cutaneous anaphylaxis. And in this case, it is IgG2 which is unable to bind; IgG1, 3 and 4 all bind.

Then finally, we have binding to human monocytes. Here IgG1 and IgG3 are effective but not IgG2 or IgG4.

<Turner to camera and then over diagram showing polypeptide structure of human IgM>

I should now like to move on to some of the very recent information which has become available on structure and function of IgM and IgE. During the last 6 months, the complete amino acid sequence of an IgM molecule has been published from two laboratories, Hilschman in Germany and Putnam's in America. And the next chart here shows not the amino acid sequence but the gross polypeptide structure, which we can now propose on the basis of these sequence studies.

And the interesting feature which I want to bring out here is that in IgM, we have an extra domain. You see here the light chains of the subunits. Here is a light chain, here is a light chain, and again here. These are conventional light chains with a variable part and a constant part. But if we look at the heavy chain, the mu chain, we have the variable region and then we have one, two, three, four constant homology regions, so in the mu chain we have an extra disulphide loop. And this explains why the mu chain is, in fact, a little bit heavier than the gamma chain. If we isolate the mu chain by chromatography, it can be shown to be heavier than the gamma chain. And it is because there is this extra 110 amino acid residues.

What is not clear at the moment is which of these three domains in the Fc region is the extra one. We don't know either which of the three is responsible for complement fixation in the case of IgM. But what is clear from the structure published so far is that polymerisation takes place through the second domain, here, so the disulphide bridges between the different subunits in the pentamer occur between the cysteine in the second domain.

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<Turner to camera and then over diagram of mast cell>

The other immunoglobulin which is attracting a great deal of interest at the moment, both from a structural point of view and functional point of view, is IgE. And the availability of myeloma proteins of IgE class since 1967 has made an enormous amount of difference to our understanding of the mechanisms of immediate hypersensitivity. And here we see the current view for the mechanism of immediate hypersensitivity at the molecular level. We know that the IgE antibody is able to bind to the surface of tissue mast cells and circulating basophils. And this binding is thought to take place through the Fc regions of the molecule.

<Turner to camera>

Now, in healthy non-atopic individuals, there are probably of the order of 20 000 IgE molecules per circulating basophil, but in individuals with a particular form of atopy or asthma, the concentration of IgE molecules on the surface of the basophil can rise to 40 000 per cell. And if one has two IgG molecules adjacent to each other with the same combining specificity in their Fab regions for a particular antigen or allergen, a bridging reaction can take place, as shown by this structure here, which represents an allergen. And this sets in motion some multi-enzyme process which results in the release of vasoactive amines from the granules of the mast cell. And three of these vasoactive amines have been characterised in some detail, all have molecular weights of less than 1000, and all are known to be released from the cell without actually causing any damage to the cell. These are histamine, slow reacting substance-A, and the eosinophil chemotactic factor. And these are the substances that are responsible for many of the symptoms of immediate hypersensitivity reactions.

At the molecular level, we are still very unsure about the triggering mechanism which takes place when the allergen combines with the IgE antibody. Stanworth has suggested that there may be a cluster of basic amino acid residues in the Fc part of

the IgE molecule, which delivers a pulse of positive charge to the cell when the antibody is conformationally altered by the bridging reaction shown here. In other words, that there must be a second site in the IgE molecule which is going to interact with the cell.

Clearly, the first site, the tissue-binding site is present on all IgE molecules of all individuals. Even non-atopic individuals have got IgE molecules on the surface of their mast cells and basophils, but they do not fire off the release of these vasoactive amines here. Perhaps, this is mainly because the concentration of antibodies with the same binding specificity in the Fab region is not sufficient to bring two molecules close to each other.

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<Turner to camera and then over diagram of polypeptide structure of human IgE>

Work at the moment on IgE is concerned with trying to define the location in the molecule of these sites which may interact with the mast cell surface. And this chart here shows the most up-to-date view we have of the IgE molecule based on sequence studies from Bennich at Uppsala. And this shows a four-chain structure that we've become used to, with two light chains out here bonded to the epsilon heavy chains between the variable region and the constant region.

There seems to be good evidence already that IgE, in common with IgM, has an extra domain and so we can talk of four constant region domains, from Cε1 through to Cε4. And to date, various fragments of the IgE molecule have been obtained by peptic digestion and papain digestion, and tested for their ability to block the immediate hypersensitivity reaction. And so far, only the whole epsilon chain for an Fc fragment, starting from about here and including the whole of these three domains, have been effective in this type of blocking reaction, so we are still a long way from actually localising the site in the IgE molecule which binds to the mast cell surface, but from analogy to the monocyte binding site of human IgG, we might

postulate that the cell-binding site of IgE would logically be located in this Cε4 domain in here.

One interesting feature before we leave this chart is that Bennich has proposed a disulphide bridge between the epsilon chains just where the variable regions end. And if this is confirmed, this puts a considerable constraint on the degree to which this immunoglobulin molecule can open to accommodate to the two antigen-binding regions. In other words, the ability of the Fab paths of the molecule to swing away from each other and to bind to the allergen is considerably restricted by this disulphide bridge here. Most immunoglobulins have their bridges in the middle of the molecule here and this enables a fair amount of flexibility between the two Fab regions. And this may relate to the fact that many allergens have been reported to consist of repeating antigenic structures, or to be, at least, to have contained divalent antigenic structures.

<Turner to camera>

Well, it is often said that the immunoglobulins represent one of the most solid parts of our knowledge of immunology and to some extent this is true. In addition to the complete amino acid sequence of IgG and IgM, we shall soon have a complete sequence for IgE. But, on the other hand, we know very little about the structure of IgA and practically nothing about the structure of IgD. And perhaps most important of all, we need to learn a great deal more about the function, physiology and life history of all of these proteins.

<End credits>